

Using fruit-bearing cuttings of grapevine and temperature gradient greenhouses to evaluate effects of climate change (elevated CO₂ and temperature, and water deficit) on the cv. red and white Tempranillo. Yield and must quality in three consecutive growing seasons (2013–2015)

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ABSTRACT

In the Mediterranean area, changes in crop production and quality are expected in the future, due to one or more stress factors associated with climate change. Among them, plant responses to atmospheric CO₂ concentration increases, enhanced temperatures and scarce water availability are a matter that deserves further investigation. In this study, the effects of the three above-mentioned factors, acting individually and/or in interaction, on grapevine reproductive growth and berry quality were investigated in three consecutive growing seasons (2013, 2014, and 2015) in the cultivars red and white Tempranillo. Eight different treatments were applied from fruit set to maturity (2 CO₂ levels (400 versus 700 μmol mol⁻¹) × 2 temperature treatments (ambient versus ambient +4 °C) × 2 water availability regimes (well irrigated versus cyclic drought)) in four temperature gradient greenhouses located at the University of Navarra (Pamplona, Spain). Yield was significantly reduced by drought and was year-dependent. Eventual heat shocks (above 35 °C) in the first week of July in 2015 induced berry burn, browning and loss of 50% of the berries. Regarding quality, simulated climate change scenarios affected to greater extent technological (primary metabolism) than phenolic (secondary metabolism) maturity. Indeed, high temperature and drought significantly and consistently increased must pH, due to decreases in malic acid. On the contrary, elevated CO₂ decreased pH associated with significant increases in tartaric acid. Differences in the response of red and white Tempranillo were found. Acidity was lower (and pH higher) in white than in red Tempranillo, due to lower malic and tartaric acid concentrations. Also, total polyphenol index was lower in the white variety, in part due to the absence of anthocyanins. Fresh bunch weight and berry water content were higher in the white than in the red variety. Interactions found among variety, year, water availability, temperature and CO₂ have been highlighted and discussed.

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1. Introduction

The most important anthropogenic greenhouse-effect gas, atmospheric carbon dioxide (CO₂), has risen since pre-industrial

period until nowadays from 280 to more than 400 μmol mol⁻¹ air (ppm), and it is foreseen to reach ca. 700 ppm at the end of this century. Due to continuous and intense CO₂ emissions and its greenhouse effect, models projected an average temperature increase of between 1.8 and 4.0 °C by the year 2100. Moreover, plant water availability is predicted to be lower by that time, intensifying the arid agricultural areas and impacting crop production (Collins and Bras, 2007). The Mediterranean area is particularly very vulnerable to a temperature and aridity increase, as well as to sustained severe weather (Tubiello et al., 2000). Therefore, climate-related factors such as elevated CO₂, elevated temperature and altered

Abbreviations: DW, dry weight; FW, fresh weight; IPCC, intergovernmental panel on climate change; T, ambient temperature; T + 4, 4 °C more than ambient temperature; WA, water availability; FI, full irrigation; CD, cyclic drought; TGGs, temperature gradient greenhouses.

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water availability will affect crops growing in the Mediterranean area in the future.

Effects of atmospheric CO₂ concentration have been investigated in a variety of agricultural crops (Cure and Acock, 1986), legumes (Irigoyen et al., 2014), trees (Ceulemans and Mousseau, 1984) and other plant species (Idso and Idso, 1994; Poorter, 1993), including grapevine (Schultz, 2000). Climate change is exerting an increasingly profound influence on grapevine phenology (Martínez-Lüscher et al., 2016a, 2016b; Salazar Parra et al., 2010) and grape composition (Gonçalves et al., 2009; Kizildeniz et al., 2015; Salazar Parra et al., 2010). Grape affected phenolics by climate change include anthocyanins (Salazar Parra et al., 2010; Torres et al., 2016) and flavonoids (Pastore et al., 2017; Torres et al., 2017). Ultimately, these changes affect vinification process (Jones and Davis, 2000; Mira de Orduña, 2010).

A common response to elevated CO₂ is an increased grapevine plant growth and yield (Bowes, 1993). Only some grape constituents are transiently influenced by elevated CO₂ during berry development, whereas others are not affected. In particular, (i) must acids and sugars were positively impacted at different stages of berry growth, although effects vanished (Bindi et al., 2001) or not (Kizildeniz et al., 2015) at maturity, and (ii) must total anthocyanins and tannins (Gonçalves et al., 2009; Kizildeniz et al., 2015), and color intensity and tonality (Salazar Parra et al., 2010) were not modified.

High temperature has a huge impact on enzyme activities. In grapevine berries, elevated temperature affects anthocyanin accumulation through disturbing several enzymes of the anthocyanin metabolic pathway (Cohen et al., 2008; Yamane et al., 2006), resulting in lower anthocyanin contents (Kliewer, 1970; Mori et al., 2007; Torres et al., 2016). Grapevine elevated temperature experiments also indicate that the grape must acidity was decreased, affecting especially grape malic acid concentration (Spayd et al., 2002; Sweetman et al., 2014). For that reason, cooler regions are generally producing grape berries with higher malic acid levels than warmer regions (Blouin and Guimberteau, 2003). Recently, Torres et al. (2016) reported that temperature decreases grape malic acid concentration in some clones but not in others of red Tempranillo.

Soil water availability has marked negative impact on grapevine vegetative growth (Kliewer and Weaver, 1971; Matthews et al., 1987; Matthews and Anderson, 1989; Williams and Matthews, 1990), generally decreasing moreover berry yield and quality (Myburgh, 2003). Berry size decreases if grapevine faces water stress during early phenological stages, due to restrictions in cell division (Matthews and Anderson, 1989; McCarthy, 1997; Ojeda et al., 2002). As a consequence, water stress may impact positively, through a depression in berry size, berry quality indirectly via concentration of must phenolics, including anthocyanins (Castellarin et al., 2007; Matthews et al., 1990; Petrie et al., 2004). However, other effects of water stress on berry quality reported in the literature are rather incoherent, possibly related to the phenological phase in which water stress occurs, or to the intensity and duration of the water deficit. Thus, (i) increases in malic acid concentration (López et al., 2007), sugar content (Antolín et al., 2006; Koundouras et al., 2006; Matthews et al., 1990; Ojeda et al., 2002) and pH values (Bahar et al., 2011), (ii) decreases in malic acid concentration (De Souza et al., 2005; Intrigliolo and Castel, 2009; Koundouras et al., 2006; Salazar Parra et al., 2010), berry volume, total soluble solids, potassium and tartaric acid concentration (Bahar et al., 2011) or (iii) no change on malic acid concentration (Esteban et al., 1999) have been reported in response to water stress. With respect to phenolic compounds, a recent report shows that anthocyanins and flavonoids were mainly affected by timing of water deficit throughout ripening (Torres et al., 2017).

For all the above-mentioned reasons, performing greenhouse experiments under controlled conditions (including a fine control

of CO₂ concentration, temperature, and water availability) and with several repetitions could help to gain information about the effects of foreseen climate change on grapevine yield and berry quality. Different growth systems, such as open top chambers (OTC), free air CO₂ enrichment (FACE) systems, temperature gradient greenhouses (TGGs) and others, have been used to investigate the effects of elevated CO₂ on plants (Morales et al., 2014). In the present work, TGGs were used for their advantages as research-oriented greenhouses, allowing us to investigate the effects of elevated CO₂, elevated temperature and water availability separately or in interaction (as multiple stress factors). Although the effects of these climate change-related factors as single stress factors have been investigated (see above), their interactions were rarely studied. Combination of all these factors caused more severe effects on berry weight (McCarthy, 1997), and changes in berry quality were more complex (Schultz, 2000; Kizildeniz et al., 2015). Elevated CO₂ attenuated the negative effects of temperature (Bindi et al., 2001) or drought (Kizildeniz et al., 2015) on grapevine production. In the present study, we investigated the effects of elevated CO₂, elevated temperature and water deficit (either acting individually or interacting among them) on yield, and must quality in fruit-bearing cuttings of two grapevine (*Vitis vinifera* L.) cultivars (red and white Tempranillo) during 3 years (2013, 2014, and 2015). No many works have been reported using the TGGs and fruit-bearing cuttings of red (Kizildeniz et al., 2015; Salazar-Parra et al., 2015) or white (Kizildeniz et al., 2015) Tempranillo. The Salazar-Parra et al. (2015) report did not deal with grape quality. In all those cases, experiments reported lasted one year, in contrast to the multi-year approach of this report.

2. Materials and methods

2.1. Plant material

Dormant cuttings of *Vitis vinifera* L. cv. red (accession T43, clone RJ-43) and white Tempranillo (accession CI-101 in “La Grajera” germplasm bank, Riojaís Government, Spain) were sampled from an experimental vineyard of the Institute of Sciences of Vine and Wine (ICVV) in Logroño (La Rioja, Spain). Experiments were conducted using each year new cuttings (not imposing treatments on the same plants for 3 consecutive years). In this work, we used own-rooted plants. In the past, we tried to adapt the technique of fruit-bearing grapevine cuttings to grafted varieties. It was unsuccessful. We do believe that the resources driven to form the graft are high, not allowing the proper and normal development of the bunch.

Tempranillo is a local red grape variety widely cultivated in northern and central Spain, currently expanded to many other viticulture areas in the world. A new white berry somatic variant of Tempranillo (“white Tempranillo”) is the result of a spontaneous, natural mutation, which was discovered in an old red Tempranillo vineyard in 1988 in Murillo de Río Leza, La Rioja, Spain (García-Escudero et al., 2011) and vegetatively propagated from one single grapevine plant.

2.2. Growth conditions

Fruit-bearing cuttings were obtained from dormant cuttings according to Mullins (1966), as described by Kizildeniz et al. (2015). Briefly, cuttings were treated with indole butyric acid (300 mg L⁻¹) in a heated rockwool moist-bed (25–27 °C) kept in a cool chamber (5 °C). One month later, the rooted-cuttings were planted in 0.8 L plastic pots containing a mixture of sand, perlite and vermiculite (1:1:1, in volume) and transferred to the greenhouse. Only a single flowering stem was allowed to develop on each

plant, in order to get only one berry bunch per plant. Until fruit set, vegetation was controlled and only 4 leaves per plant were allowed to grow. At fruit-set, the cuttings were transplanted to 13 L plastic pots containing a peat and perlite (2:1, v/v) mixture. During growing period, the greenhouse was set at 26/15 °C and 60/80% relative humidity (RH) (day/night) and photoperiod of 15 h with natural daylight supplemented with high-pressure metal halide lamps (OSRAM®, Augsburg, Germany), providing a photosynthetic photon flux density (PPFD) of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at inflorescence level. Nutrient solution was applied as proposed by Ollat et al. (1998): NH_4NO_3 (64.5 mg L^{-1}), $(\text{NH}_4)_2\text{HPO}_4$ (75 mg L^{-1}), KNO_3 (129 mg L^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (125 mg L^{-1}), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (248 mg L^{-1}), $(\text{NH}_4)_2\text{SO}_4$ (66 mg L^{-1}), Fe-EDDHA (280 mg L^{-1}), H_3BO_3 (2.86 mg L^{-1}), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.81 mg L^{-1}), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.22 mg L^{-1}), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.08 mg L^{-1}) and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (0.016 mg L^{-1}). These growing conditions were maintained until fruit set from March to May 2013, 2014 and 2015.

2.3. Temperature gradient greenhouses and experimental design

Treatments' application was made in four temperature gradient greenhouses (TGGs), located in the campus of the University of Navarra (42°48'N, 1°40'W; Pamplona, Navarra, Spain), from June to August 2013, 2014 and 2015 (fruit set to maturity). It is evident that the TGGs used in this work move plants away from their growing natural conditions. Quantifying this in scenarios of climate change is difficult because plants in the field face multiple stress factors. However, we do believe that TGGs are a good approach, because TGGs are a valid way of ascribing to one specific factor, CO_2 , temperature or water availability, a physiological response, enabling for mechanistic researches on the causes of the physiological responses to future climate conditions. Readers are referred to Morales et al. (2014, 2016) for further discussion about differences between results obtained from vineyards and the fruit-bearing cutting technique.

TGGs have a design based on temperature gradient tunnels (Rawson et al., 1995), which allows investigating the effects on plants of environmental changes, such as elevated temperature, elevated CO_2 and drought, acting separately or in combination. TGGs contain 3 temperature modules (3.04 m long each), CO_2 can be injected inside increasing the air CO_2 concentration as desired, and a gradient of temperature is created along them (from module 1 of ambient temperature to module 3 of ambient +4 °C) (Morales et al., 2014). Also, plants growing inside the TGGs can be subjected to different levels of irrigation. Thus, TGGs are used as research greenhouses with high level of environmental control and with plants in pots growing inside (Morales et al., 2014). In the TGGs, CO_2 concentration, temperature, relative humidity and radiation were monitored and/or controlled by an informatics system. The treatments applied to the two grapevine varieties (red and white Tempranillo) were a combination of two CO_2 levels (ambient ca. 400 ppm, and elevated ca. 700 ppm), two temperature regimes (ambient and ambient +4 °C) and two water availability conditions (full irrigation and cyclic drought). Water treatments were applied and substrate water content recorded according to Kizildeniz et al. (2015).

2.4. Measurements and analyses

Berry fresh weight (FW) was measured, and then oven-dried at 80 °C for 48 h in order to obtain DW (or dry mass (DM)). Berry water content was calculated as $(\text{FW} - \text{DW})/\text{DW}$.

Harvested grapevine berries (21–23°Brix) were frozen in liquid nitrogen and stored at –80 °C until analysis before being oven-dried. Samples of 30 frozen berries per biological replicate (each biological replicate consisted of a pool of berries from three dif-

ferent plants) were peeled, extracted and centrifuged for 10 min at 4302 $\times g$ (Sorvall SS-34 rotor, 6000 rpm) and the supernatants utilized for technological maturity determinations. Must total soluble solids (°Brix) was assessed with a refractometer (Zuzi model n°315, Digital ABBE). Potassium was measured by flame emission spectroscopy. Must pH was evaluated with pH meter (Microph Cricson model 2000). The modified Rebelein's method was applied for quantifying tartaric acid, determined spectrophotometrically (Model U-2001, Hitachi Instruments Inc., USA) at 530 nm (Rebelein, 1973; Vidal and Blouin, 1978). L-Malic acid was determined applying an enzymatic method, the L-malic acid test kit (Enzytec™ L-Malic Acid, Boehringer Mannheim/R-Biopharm).

A second sample of 30 berries per biological replicate was weighed and ground in blender. It was centrifuged for 10 min at 4302 $\times g$ (Sorvall SS-34 rotor, 6000 rpm) and the supernatant was removed to another tube for the determination of color density and tonality (hue). Color density was the result of the sum of the optical densities at 420, 520, and 620 nm, measured with 1 cm optical path (Glories, 1978). Phenolic maturity was evaluated after homogenate maceration during 4 h according the Glories' method (Glories and Augustin, 1993). In brief, homogenate and buffer were mixed 1:1 by volume. The buffer was set at pH 1, extracting total anthocyanins. Subsequently, the macerated samples were centrifuged for 10 min at 4302 $\times g$ (Sorvall SS-34 rotor, 6000 rpm), and the supernatants used for the following determinations. Total anthocyanin concentration was determined spectrophotometrically according to Ribéreau-Gayon and Stronestreet (1965). Total polyphenol index (TPI) was measured in the supernatant obtained after maceration at pH 3.2 (diluted 100 times with distilled water) at 280 nm according to Ribéreau-Gayon et al. (1972). Tonality was measured as the 420/520 nm optical densities ratio in the supernatant obtained after maceration at pH 3.2 (Salazar Parra et al., 2010).

Data were analyzed with XLStat 7.5.2 Pro® statistical software (Addinsoft, Paris, France) by a factorial ANOVA. Fisher's Least Significant Difference (LSD) *post-hoc* test was used for means comparison. Results were considered statistically significant at $P < 0.05$. Only main factors and two-level interactions P-values were considered. For simplicity, only those two-level interactions that were significant in at least one trait are shown throughout the manuscript.

3. Results

3.1. Foreseen climate change, yield and berry water content in V. vinifera cv. red and white Tempranillo

Considering the three years of experiments, white Tempranillo yielded more than the red one (Fig. 1, Table S1; $P_{(\text{Variety})} = 0.003$). Differences reached 8% in bunch FW (Table S1) and were caused mostly to the behavior of the varieties during the year 2015 resulting in a highly significant interaction between variety and year (Fig. 1, Table S1; $P_{(\text{Variety})} \times P_{(\text{YEAR})} = 0.0001$). Grapevine yield was negatively impacted by water stress, causing a 14% reduction in bunch FW (Fig. 1, Table S1; $P_{(\text{WA})} = 0.0004$). Most of these changes occurred in 2014, data giving a significant interaction between water availability and year (Fig. 1, Table S1; $P_{(\text{WA})} \times P_{(\text{YEAR})} < 0.05$). Contrarily to water stress, elevated CO_2 increased 14% yield (Fig. 1, Table S1; $P_{(\text{CO}_2)} = 0.003$). Globally, during the 3 years of experiments, elevated temperature had no significant effect on yield directly (Fig. 1, Table S1). However, heat shocks occurring in July 2015 caused an important yield loss, reducing largely the grapevine bunch FW of both red and white Tempranillo (Fig. 1).

Berries of white Tempranillo plants had 12% higher water content than those of the red variety (Fig. 2, Table S1; $P_{(\text{Variety})} < 0.0001$). Elevated CO_2 , on the contrary, decreased 8% berry water content

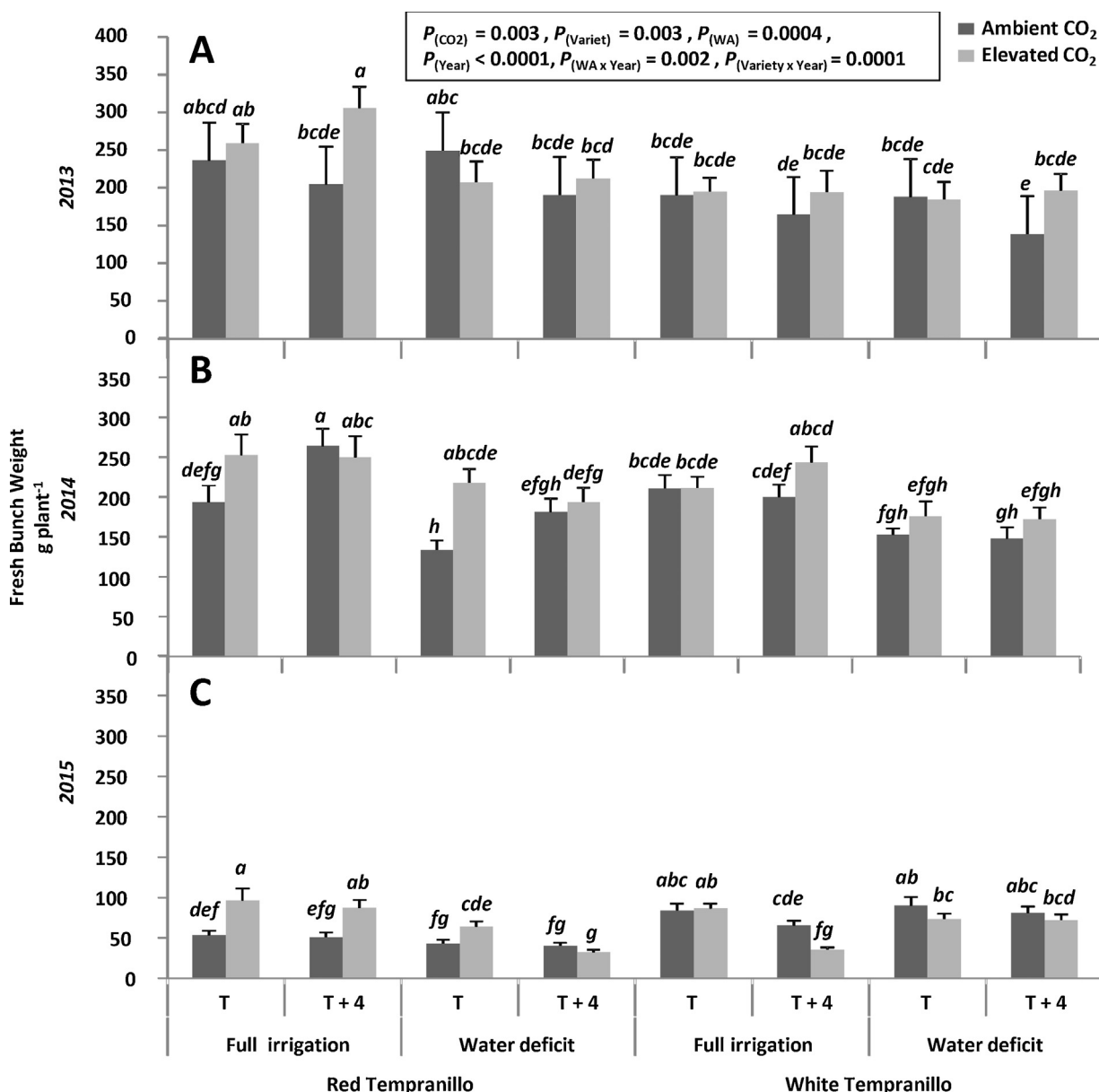


Fig. 1. Fresh bunch weight (g plant^{-1}) recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high ($T+4^\circ\text{C}$) or ambient (T) and irrigation treatments: full irrigation or water deficit (cyclic drought) during 2013 (A), 2014 (B) and 2015 (C). Values represent means ($n=8-10$). Within each parameter and year, means followed by different letters are significantly different ($P < 0.05$) based on LSD test.

(Fig. 2, Table S1; $P_{(CO_2)} = 0.009$). Neither elevated temperature nor water availability impacted berry water content (Fig. 2, Table S1).

3.2. Foreseen climate change and technological maturity in *V. vinifera* cv. red and white Tempranillo

Bunches of all plants were harvested at maturity (defined as $>21-23^\circ\text{Brix}$ for Tempranillo), sampling some berries from the biological replicates and using their $^\circ\text{Brix}$ values to choose harvest time. However, the range of values found at maturity in the whole bunch of berries varied slightly (Table 1). Data revealed that the TSS ($^\circ\text{Brix}$) of white Tempranillo was 2.1% higher than that of red (Table 1). Also, the concentration of TSS increased 2.6% when plants were grown under elevated CO_2 when the whole data were analyzed, reaching 5.3% in droughted plants (Table 1).

Malic acid is one of the main organic acids present in grapevine berries. White Tempranillo berries had 10% less malic acid than

the red ones (Fig. 3, Table S1; $P_{(Variety)} = 0.001$). According to the three experimental repetitions, its concentration in the berries decreased significantly when plants were grown under elevated temperature ($P_{(T)} < 0.0001$; 11%), elevated CO_2 or water deficit ($P_{(CO_2)}$ and $P_{(WA)} = 0.02$ and 7%, in both) (Fig. 3, Table S1). All the above-mentioned effects were year dependent ($P_{(Year)} < 0.0001$), showing interaction with temperature ($P_{(Temp \times Year)} = 0.01$), CO_2 ($P_{(CO_2 \times Year)} = 0.01$) and variety ($P_{(Variety \times Year)} = 0.0002$) (Fig. 3, Table S1).

The other main organic acid component in grape berries is tartaric acid. As in the case of malic acid, white Tempranillo berries had less (17%) tartaric acid than those of the red variety (Fig. 4, Table S1; $P_{(Variety)} = 0.0004$). These differences depended on the year (Fig. 4, Table S1; $P_{(Variety \times Year)} = 0.005$). Water availability had no impact on grape tartaric acid concentration, whereas elevated CO_2 ($P_{(CO_2)} = 0.0003$; 20%) and elevated temperature ($P_{(Temp)} = 0.03$; 12%) increased it significantly (Fig. 4, Table S1).

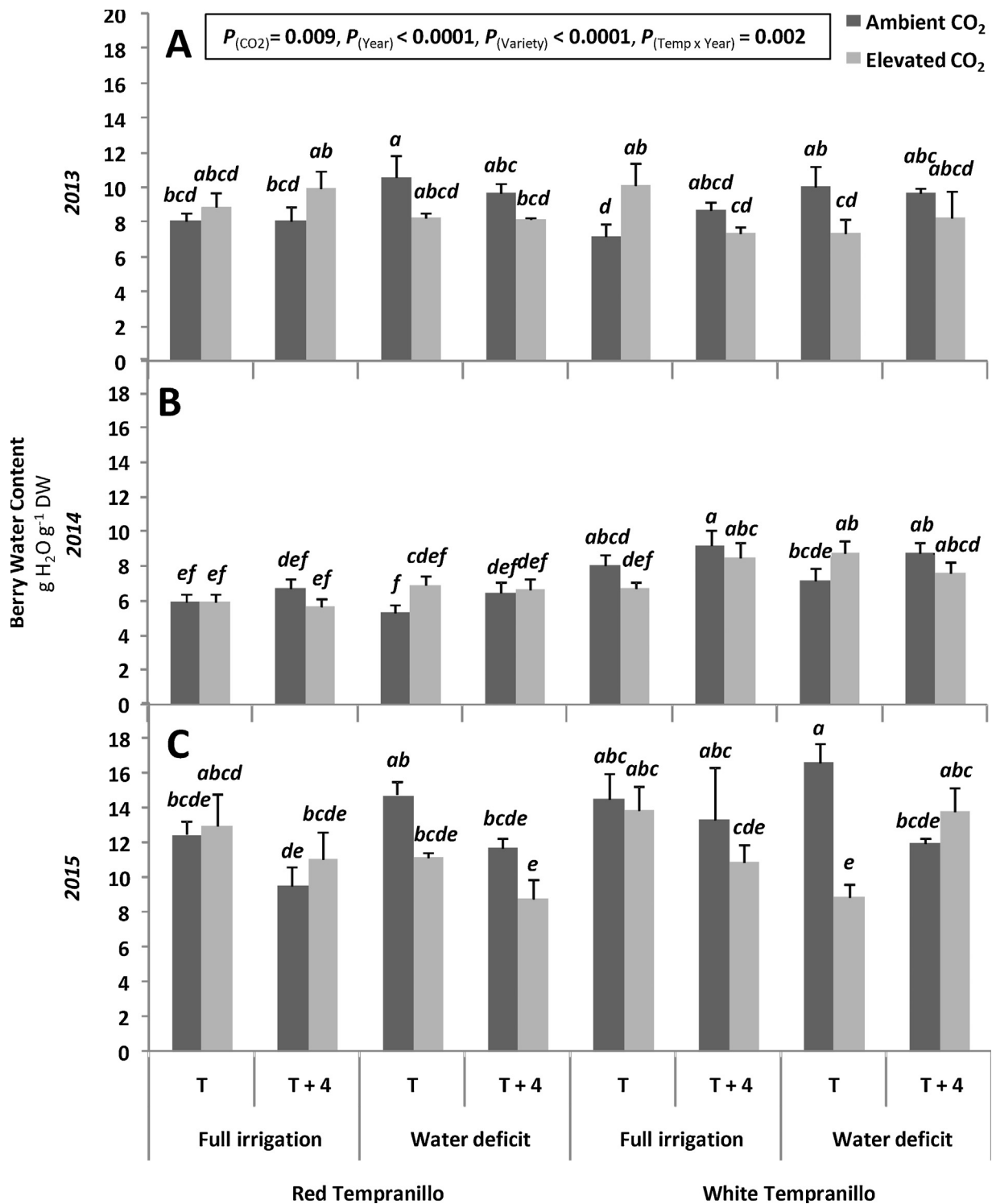


Fig. 2. Berry water content (g H₂O g⁻¹ DW) recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO₂ levels: elevated CO₂ (700 ppm) or ambient CO₂ (400 ppm), temperature regimes: high (T+4°C) or ambient (T) and irrigation treatments: full irrigation or water deficit (cyclic drought) during 2013 (A), 2014 (B) and 2015 (C). Values represent means (n = 3–10). Within each parameter and year, means followed by different letters are significantly different ($P < 0.05$) based on LSD test.

As a consequence of the differences in malic and tartaric acid concentrations, musts from white Tempranillo berries had less acidity (11%, $P < 0.0001$) and higher pH (1.4%, $P < 0.05$) than musts obtained from the red berries (Table 1). Drought and elevated temperature decreased must acidity (10 and 12%, respectively) and increased pH (4.2 and 2.8%, respectively) (Table 1, $P < 0.0001$ in all cases). Water scarcity increased must pH more in red (5.7%) than in

white (2.7%) Tempranillo (Table 1). On the contrary, elevated CO₂ decreased 3.7% must pH (Table 1, $P_{(CO_2)} < 0.0001$), possibly related to the 20% increase in tartaric acid (Table S1) and 11% decrease in K (Table 1, $P_{(CO_2)} < 0.0001$) concentrations. With respect to must pH, there was a clear interaction between elevated CO₂ and temperature, and thus the extent of the CO₂-mediated decrease was larger at elevated (6%) than at current (1.2%) temperature (Table 1). Potas-

Table 1

Total soluble sugars (TSS), pH, acidity, potassium, and total polyphenol index (TPI) in red and white Tempranillo grown under different CO₂ concentrations (ambient (A_{CO2}) or 700 ppm CO₂ (E_{CO2})), temperature regimes (ambient (T) or ambient +4 °C (T+4)) and water availability (full irrigation (FI) or cyclic drought (CD)). Data were plotted as minimum (Min.) and maximum (Max.) means (n=9) and analysis of variance (ANOVA) P values of mean effects of variety (P_(VAR)), water availability (P_(WA)), temperature (P_(Temp)), CO₂ (P_(CO2)), year (P_(Year)) and their two level interactions among them are shown. *, P<0.05; **, P<0.001; ***, P<0.0001 and P>0.05, n.s. not significant. The percentages of change shown are means (n=30) of three years (2013, 2014 and 2015). Arrows indicates increment (↑) or reduction (↓) with respect to control treatments (red Tempranillo was taken as control for comparisons of white Tempranillo).

Treatments				TSS ° Brix	pH	Acidity g Tartaric acid L ⁻¹ Min.–Max.	Potassium mg L ⁻¹	TPI A _{208 nm}		
Red	FI	T	A _{CO2}	20.7–22.6	3.8–4.0	3.9–5.4	2111.7–3113.6	40.1–63.1		
			E _{CO2}	21.0–22.7	3.5–4.0	4.8–5.0	2557.7–3260.8	28.8–58.7		
	CD	T + 4	A _{CO2}	21.6–22.5	3.9–4.1	4.1–4.5	1886.0–3334.3	41.5–58.9		
			E _{CO2}	21.3–23.0	3.8–4.0	3.9–5.1	1946.2–2568.6	42.3–59.2		
		T	A _{CO2}	21.8–22.0	3.8–4.2	3.9–4.9	2473.5–3713.7	48.1–89.5		
			E _{CO2}	22.3–24.7	4.0–4.2	3.7–4.4	2237.9–3493.2	40.8–58.2		
	CD	T + 4	A _{CO2}	20.8–23.3	4.3–4.5	3.6–4.7	1796.6–3326.7	44.5–71.5		
			E _{CO2}	20.2–22.7	3.9–4.0	3.3–5.0	1813.5–2312.1	19.7–71.1		
		FI	T	A _{CO2}	20.3–22.6	3.9–4.0	3.4–5.4	2205.4–3231.3	41.1–54.2	
				E _{CO2}	19.0–23.8	3.9–4.1	3.5–5.5	2256.6–2965.5	44.2–50.5	
	White	CD	T + 4	A _{CO2}	20.1–22.2	3.9–4.2	3.1–3.7	2372.0–2823.5	34.4–44.3	
				E _{CO2}	21.0–21.7	3.8–4.0	3.1–4.9	1694.5–2933.2	32.5–43.1	
T			A _{CO2}	19.0–22.3	4.0–4.1	3.4–5.2	2439.1–3198.5	45.3–68.7		
			E _{CO2}	22.8–23.5	3.8–4.1	3.0–5.2	2488.7–2802.8	43.9–66.9		
CD		T + 4	A _{CO2}	19.3–22.0	4.2–4.6	2.4–4.5	1928.6–2634.4	33.2–49.5		
			E _{CO2}	21.5–23.3	4.0–4.1	2.3–5.0	2380–2606.0	31.7–45.7		
		ANOVA P of main factors			P(VAR)	* (2.1%) ↑	* (1.4%) ↑	*** (10.7%) ↓	n.s.	*** (13.4%) ↓
					P(WA)	n.s.	*** (4.2%) ↑	*** (10.2%) ↓	* (6.8%) ↑	n.s.
			P(Temp)	n.s.	*** (2.8%) ↑	*** (11.8%) ↓	n.s.	*** (12.9%) ↓		
			P(CO2)	* (2.6%) ↑	*** (3.7%) ↓	n.s.	*** (11.4%) ↓	* (9.6%) ↓		
			P(Year)	***	n.s.	***	***	***		
			P(VAR) × P(WA)	n.s.	*	n.s.	**	n.s.		
			Red*WA	n.s.	5.7% ↑	n.s.	13.4% ↑	n.s.		
			White*WA	n.s.	2.7% ↑	n.s.	0.6% ↑	n.s.		
			P(VAR) × P(Temp)	n.s.	n.s.	n.s.	n.s.	**		
			Red*Temp	n.s.	n.s.	n.s.	n.s.	3.2% ↓		
			White*Temp	n.s.	n.s.	n.s.	n.s.	22.9% ↓		
			P(VAR) × P(Year)	**	n.s.	***	***	***		
			P(WA) × P(CO2)	*	n.s.	n.s.	n.s.	n.s.		
			FI*CO2	0.1% ↓	n.s.	n.s.	n.s.	n.s.		
			CD*CO2	5.3% ↑	n.s.	n.s.	n.s.	n.s.		
			P(WA) × P(Year)	n.s.	*	**	*	***		
			P(Temp) × P(CO2)	n.s.	**	n.s.	n.s.	n.s.		
			T*CO2	n.s.	1.2% ↓	n.s.	n.s.	n.s.		
			T + 4*CO2	n.s.	6.0% ↓	n.s.	n.s.	n.s.		

sium increased significantly (7%) in musts obtained from grapes grown under drought (Table 1, P_(WA) < 0.05).

3.3. Foreseen climate change and phenolic maturity in V. vinifera cv. red and white Tempranillo

The total polyphenol index (TPI) trait also showed a varietal difference. TPI values were 13% lower in white than in red Tempranillo (Table 1). Water availability had no effect on TPI (Table 1). Both elevated temperature (P_(Temp) < 0.0001) and elevated CO₂ (P_(CO2) < 0.05) decreased TPI values (13 and 10% respectively), being the effects of elevated temperature larger in white (23%) than in red (3%) Tempranillo (Table 1).

We further analyzed the phenolic characteristics of red Tempranillo, because grapes from white Tempranillo have lost anthocyanins. In our study, the only factor that impacted the total anthocyanin concentration was the water availability, drought increased it (P_(WA) < 0.05) 11% (Table 2). Tonality (hue) was also affected by water stress. Musts obtained from grapes grown under drought had 6% higher (P_(WA) < 0.05) tonality than those grown under full irrigation (Table 2). With respect to color density, drought and elevated CO₂ increased it (P < 0.05 in both cases) 22 and 15% respectively. An interaction between water availability and temperature occurred. Thus, plants that were full irrigated and were grown under elevated temperature had 2.3% lower color density, whereas the combined action of cyclic drought and high temperature increased color density 29% (P_(WA × T) < 0.05; Table 2).

All these phenolic characteristics varied largely among years (P_(Year) < 0.0001; Table 2).

Ratio of total anthocyanins to TSS was highly dependent on year (P_(Year) < 0.0001), reaching values of ca. 10–20, 20–30 and 30–40 in 2013, 2014 and 2015 respectively (Fig. 5). No changes or no consistent effects were observed in response to temperature, CO₂ or water availability (Fig. 5). Temperature did not change this ratio in 2013 and 2014. In 2015, when compared to current temperature, elevated temperature decreased it in plants well irrigated and grown under current CO₂ but increased it in those grown under drought and elevated CO₂ (Fig. 5). In 9 out of 12 comparisons, elevated CO₂ did not impact the total anthocyanins to TSS ratio (Fig. 5). Generally, water stress either did not change or, in some cases, decreased the ratio (Fig. 5). The only significant interaction we observed was that of water availability with year (P_(WA × Year) < 0.0001; Fig. 5). In 2013 results were not clear enough, but the total anthocyanins to TSS ratio tended to decrease when drought and elevated CO₂ were combined, irrespective of temperature (Fig. 5). In 2014, drought decreased the ratio always, irrespective of temperature and CO₂ concentration (Fig. 5). Finally, in 2015, the ratio was decreased when drought was combined with elevated CO₂ when plants were grown at current temperature (Fig. 5). The only exception was the combination of elevated CO₂, elevated temperature and drought in 2015, which increased the ratio when compared with the well irrigated plants grown at elevated CO₂ and elevated temperature (Fig. 5).

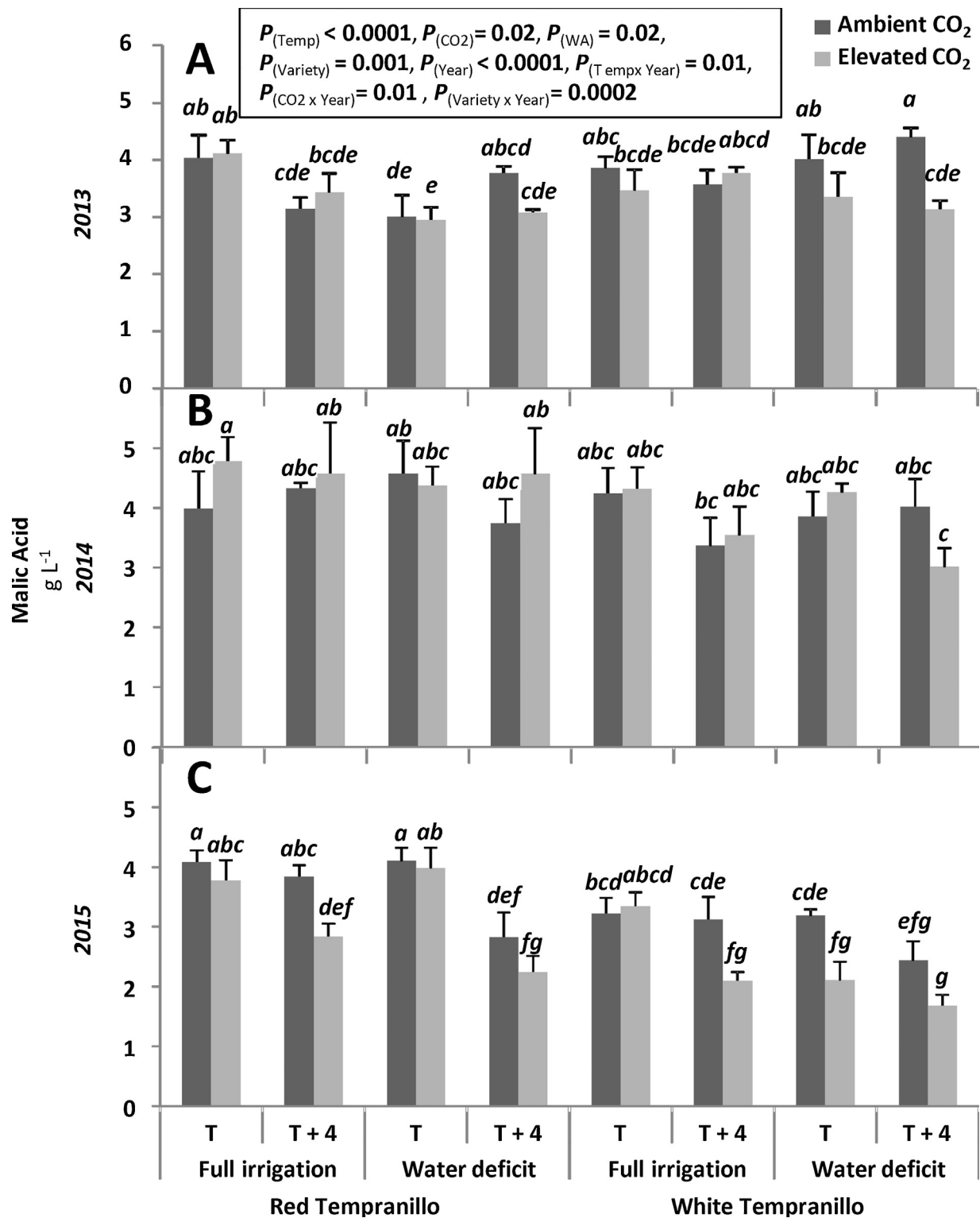


Fig. 3. Malic acid (g L^{-1}) recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO₂ levels: elevated CO₂ (700 ppm) or ambient CO₂ (400 ppm), temperature regimes: high (T + 4 °C) or ambient (T) and irrigation treatments: full irrigation or water deficit (cyclic drought) during 2013 (A), 2014 (B) and 2015 (C). Values represent means ($n = 3$). Within each parameter and year, means followed by different letters are significantly different ($P < 0.05$) based on LSD test.

4. Discussion

Changes in grapevine production and quality are expected in the future in the Mediterranean area, and in other viticulture areas, due to one or more stress factors associated with climate change. In this study, the effects of elevated CO₂, elevated temperature and

water availability, acting individually and/or in interaction, were investigated in 2013, 2014, and 2015 in the grapevine cultivars red and white Tempranillo. Among those factors, drought reduced yield in line with previous reports (Korkutal et al., 2011; Williams and Matthews, 1990) and was year-dependent, being especially low in 2015 due to eventual heat shocks in the first week of July. Grow-

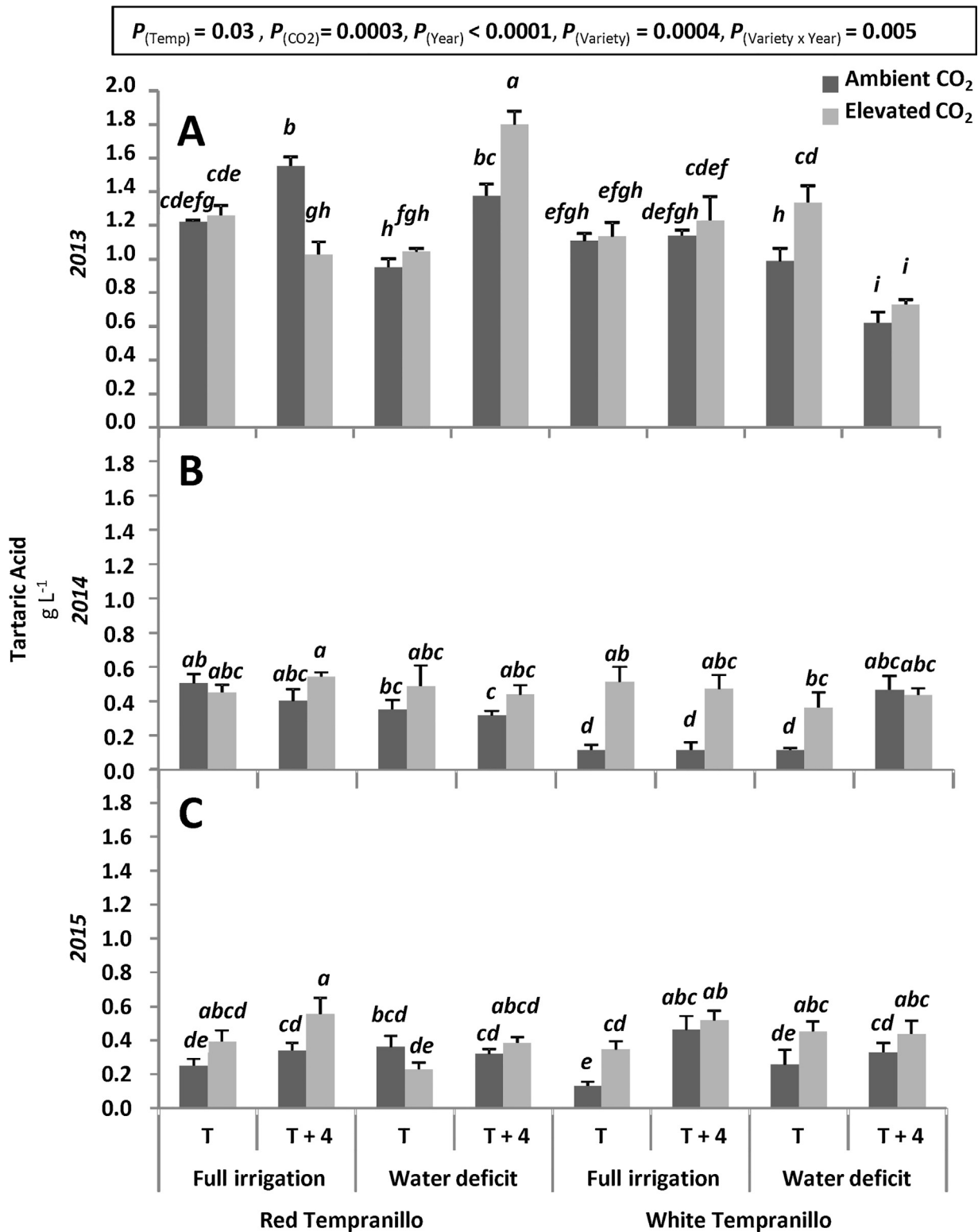


Fig. 4. Tartaric acid (g L^{-1}) recorded at harvest from fruit-bearing cuttings of red and white Tempranillo grown under two different CO₂ levels: elevated CO₂ (700 ppm) or ambient CO₂ (400 ppm), temperature regimes: high (T+4 °C) or ambient (T) and irrigation treatments: full irrigation or water deficit (cyclic drought) during 2013 (A), 2014 (B) and 2015 (C). Values represent means ($n = 3$). Within each parameter and year, means followed by different letters are significantly different ($P < 0.05$) based on LSD test.

ing under elevated temperature per se had no effect on yield but the high temperatures of the heat waves (with maximum temperatures above 35 °C for at least five consecutive days or 40 °C for three days) induced berry burn, browning and finally loss of 50% of the berries.

Grape quality changes can be indirect effects of changes in berry characteristics, in particular berry size and skin/pulp ratio. Berry weight and relative skin mass (skin DW/pulp DW) were monitored in 2013. No significant changes were observed in the relative skin mass induced by the treatments or their interactions (Mekni, 2014).

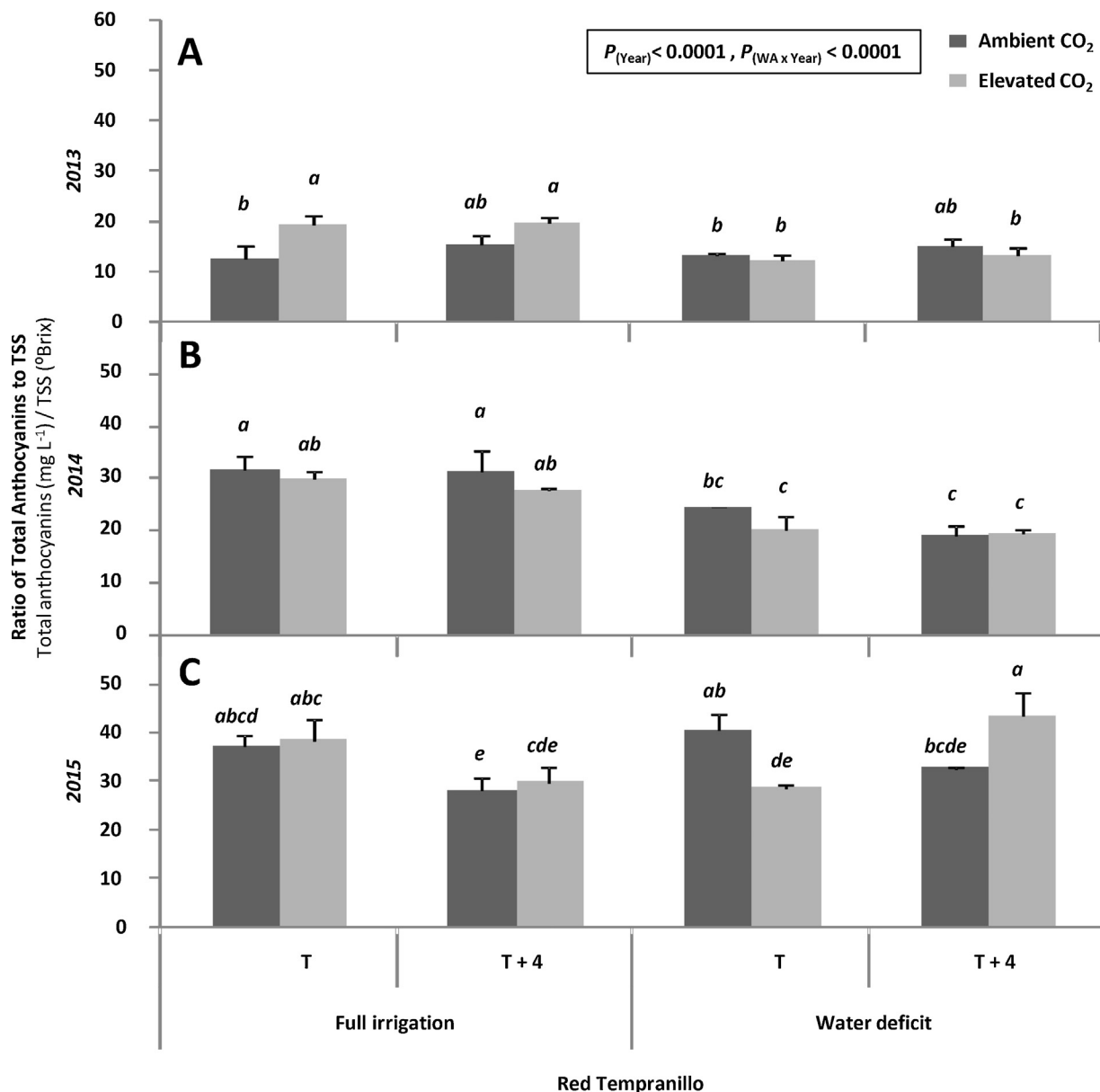


Fig. 5. Ratio of total anthocyanins (mg L^{-1}) to total soluble sugars (TSS; $^{\circ}\text{Brix}$) recorded at harvest from fruit-bearing cuttings of red Tempranillo grown under two different CO_2 levels: elevated CO_2 (700 ppm) or ambient CO_2 (400 ppm), temperature regimes: high ($T+4^{\circ}\text{C}$) or ambient (T) and irrigation treatments: full irrigation or water deficit (cyclic drought) during 2013 (A), 2014 (B) and 2015 (C). Values represent means ($n=3$). Within each parameter and year, means followed by different letters are significantly different ($P<0.05$) based on LSD test.

With respect to berry FW, no changes were recorded in response to temperature, whereas decreased when grown under elevated CO_2 with respect to ambient CO_2 (from 0.89–1.27 to 0.87–1.23 g FW; 2–3%) or increased in drought-grown plants compared to the well irrigated (from 0.89–1.20 to 0.87–1.27 g FW; 2%) (Mekni, 2014). We consider that these changes, although significant, impact scarcely in the berry quality.

Berry quality response to climate change was variable and depended on the year. Some general conclusions, however, can be reached from the three years of experimentation in the TGGs. Climate change affected in a greater extent the technological than the phenolic maturity. Grape berries have, as major organic acids, malic, tartaric, and to a lower extent, citric acid. Grape acidity depends basically on (i) the total amount of acids, (ii) the ratio of malic to tartaric acid, and (iii) the concentration of potassium (Conde et al., 2007). Acids start to accumulate during the first grape growth period, peaking before the onset of ripening (veraison).

Then, they decrease coincident with grape sugar accumulation. This dynamic is highly affected by environmental variables. In our experiments, high temperature and drought significantly and consistently increased must pH and decreased acidity, due to the decrease in malic acid. This multi-year report confirms a previous, single-year one for the same red Tempranillo variety (Salazar Parra et al., 2010). The loss of malic acid (Lakso and Kliwer, 1975; Shellie, 2006) and decreased acidity (Sadras and Moran, 2012) due to high temperatures have been attributed in controlled experiments to an increased degradation to glucose during ripening (Blouin and Guimberteau, 2003; Matthews and Anderson, 1989; Ruffner et al., 1976). In water-stressed grapevines, however, increases (López et al., 2007), decreases (Koundouras et al., 2006; Matthews and Anderson, 1989; Salon et al., 2005) or even no grape malic acid changes (Esteban et al., 1999) have been reported in the literature. On the contrary, elevated CO_2 decreased pH associated with significant increases in tartaric acid concentration. This seems to be a

Table 2

Total anthocyanins, tonality (Hue), and color density in red Tempranillo grown under different CO₂ concentrations (ambient (A_{CO2}) or 700 ppm CO₂ (E_{CO2})), temperature regimes (ambient (T) or ambient +4 °C (T+4)) and water availability (full irrigation (FI) or cyclic drought (CD)). Data were plotted as minimum (Min.) and maximum (Max.) means (n = 9) and analysis of variance (ANOVA) P values of mean effects of variety (P_(VAR)), water availability (P_(WA)), temperature (P_(Temp)), CO₂ (P_(CO2)), year (P_(Year)) and their two level interactions among them are shown. *, P < 0.05; **, P < 0.001; ***, P < 0.0001 and P > 0.05, n.s. not significant. The percentages of change shown are means (n = 30) of three years (2013, 2014 and 2015). Arrows indicates increment (↑) or reduction (↓) with respect to control treatments.

Treatments				Total Anthocyanins mg L ⁻¹ Min.–Max.	Tonality (Hue) A _{420/520 nm}	Color density Sum of A _{420,520,620 nm}				
Red	FI	T	A _{CO2}	274.9–841.2	0.61–0.88	1.38–4.74				
			E _{CO2}	406.9–870.3	0.55–0.94	1.83–4.31				
		T + 4	A _{CO2}	334.4–687.8	0.58–0.79	0.96–4.64				
			E _{CO2}	414.8–684.3	0.51–0.80	1.91–4.01				
	CD	T	A _{CO2}	287.3–879.7	0.52–0.85	1.95–3.35				
			E _{CO2}	303.4–636.7	0.59–1.01	1.37–6.92				
		T + 4	A _{CO2}	344.8–760.1	0.53–1.01	1.85–6.72				
			E _{CO2}	267.2–971.0	0.58–1.01	2.11–6.75				
			ANOVA <i>P</i> of main factors				P(WA)	*(10.7%) ↑	*(6.2%) ↑	*(21.7%) ↑
							P(Temp)	n.s.	n.s.	n.s.
				P(CO2)	n.s.	n.s.	*(14.6%) ↑			
				P(Year)	***	***	***			
ANOVA 2 level interactions				P(WA) × P(Temp)			n.s.	n.s.	*	
				FI*Temp			n.s.	n.s.	2.3% ↓	
				CD*Temp			n.s.	n.s.	28.8% ↑	
				P(WA) × P(Year)			*	*	*	

specific effect of the elevated CO₂ that would increase the tartaric acid biosynthesis and/or would decrease its degradation, evolving in opposite direction to the acidic dynamic of the grape during ripening. Water stress had no impact on grape tartaric acid, in line with previous results in red Tempranillo (Salazar Parra et al., 2010). These changes in the acidic properties of the must affect its quality and potentially that of the resulting wine through changes in the wine stability and tart taste (Conde et al., 2007).

The relationship among pH, acidity and potassium is rather complex. Free tartaric acid and its potassium salt constitute an important trait for wine quality and longevity, because it impacts must pH and wine stability (Kliewer, 1965b, 1966; Ribéreau-Gayon et al., 2006). In the grape must, potassium binds tartaric acid, reducing acidity (Kliewer, 1965a), leading to tartrate precipitation during fermentation and wine ageing (Conde et al., 2007), and resulting in low quality wines. On the other hand, high berry potassium levels may decrease the malate degradation rate (Etienne et al., 2013). In our experiments, potassium increased and decreased in musts obtained from grapes grown under drought and elevated CO₂, respectively. Changes in potassium concentration may have contributed at least in part to the observed pH changes in those treatments.

Together with sugars and acids, phenolic compounds are the most abundant constituents present in grapes that are very important for wine quality (Downey et al., 2006; He et al., 2010; Kuhn et al., 2014). Among them, anthocyanins, synthesized via the flavonoid pathway in the red varieties, play an essential role in the grape and wine color. Grape total anthocyanins decreased by the impact of drought, which agrees with previous reports working with red Tempranillo (Kuhn et al., 2014; Zarrouk et al., 2012). The increased tonality (hue) observed in these experiments in red Tempranillo grapes from water stressed plants agrees with previous reports (Martínez-Lüscher et al., 2014) and suggests a change in anthocyanin composition (Downey et al., 2006; Martínez-Lüscher et al., 2014). Grape TPI decreased by the impact of elevated temperature and by growing plants in presence of elevated CO₂, but with no changes in anthocyanin concentration. Phenolics other than anthocyanins caused obviously these decreases. In the case of elevated temperature, it could be ascribed to lower flavonols (Pastore et al., 2017). When analyzing TPI, no interaction between water availability and temperature was found, in line with a previous report (Bonada et al., 2015).

Grape veraison points out the start of the second growth phase of berries, which is characterized by an activation of the encoding genes for sugar import leading to a strong increase of sugars in the grape pulp, followed then by anthocyanin accumulation mainly in the skin (Conde et al., 2007; Coombe and McCarthy, 2000; Lecourieux et al., 2014). Therefore, sugar and anthocyanin accumulation appear to be highly related (Dai et al., 2014). It is generally assumed that climate change decouples sugars and anthocyanins in red grapes (Martínez-Lüscher et al., 2016a, 2016b), decreasing the anthocyanins to sugars ratio, which is widely ascribed to the increased temperature (Sadras and Moran, 2012). In our three experiments, no consistent decreases in the anthocyanins to sugars ratio were observed in response to elevated temperature, but decreases occurred in response to water stress (due to decreases in anthocyanins).

Finally, varietal differences detected between white and red Tempranillo are a key aspect of this report. White Tempranillo resulted of a spontaneous mutation of red Tempranillo that comprised chromosomal deletions and reorganizations, losing hundreds of gens. While white grapes lack at least two similar and adjacent regulatory genes due to a multi-allelic mutation (Walker et al., 2007), our experiments evidence loss of genes and/or changes in their activity or regulation related to primary and secondary metabolisms. Within the former, grape TSS increased, and acidity decreased and pH increased due to decreased malic and tartaric acid concentrations in white, when compared to red Tempranillo. Within the latter, TPI was lower in the white variety when compared to the red one, which in part at least can be due to the absence of anthocyanins. Also, bunch FW and berry water content were higher in the white than in the red variety. These findings may give clues for further research on the genetic differences between red and white Tempranillo.

5. Conclusions

The response of Tempranillo yield and quality was rather variable, probably due to the interactions between variety, year, water availability, temperature and CO₂ in traits such as bunch FW (variety × year), malic acid (temperature, CO₂ and variety × year), pH (temperature × CO₂), color density (temperature × water availability) and total anthocyanins to TSS ratio (water availability × year). Some general conclusions, however, can be listed. Within the

three climate change-related factors investigated, drought and heat shocks (in 2015, but not elevated temperature per se) decreased grapevine yield of red and white Tempranillo. Changes in berry characteristics (berry size and relative skin mass) impacted scarcely on grape quality. Climate change affected in a greater extent the technological than the phenolic maturity. Elevated temperature and drought consistently increased must pH, due to malic acid decreases. On the contrary, elevated CO₂ decreased pH associated with consistent tartaric acid concentration increases. The changes in the musts acidic properties affect its quality and potentially that of the resulting wine. Differences in the response of red and white Tempranillo are a key aspect in this report. Due to lower malic and tartaric acid concentrations, acidity was lower (and pH higher) in white than in red Tempranillo. In part due to the absence of anthocyanins, TPI was lower in the white than in the red variety. Also, bunch FW and berry water content were higher in the white than in red Tempranillo.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.agwat.2017.12.001>.

References

- Antolín, M.C., Ayari, M., Sánchez-Díaz, M., 2006. Effects of partial rootzone drying on yield, ripening and berry ABA in potted Tempranillo grapevines with split roots. *Aust. J. Grape Wine Res.* 12, 13–20.
- Bahar, E., Carbonneau, A., Korkutal, I., 2011. The effect of extreme water stress on leaf drying limits and possibilities of recovering in three grapevine (*Vitis vinifera* L.) cultivars. *Afr. J. Agric. Res.* 6 (5), 1151–1160.
- Bindi, M., Fibbi, L., Miglietta, F., 2001. Free Air CO₂ enrichment (FACE) of grapevine (*Vitis vinifera* L.): II: Growth and quality of grape and wine in response to elevated CO₂ concentrations. *Eur. J. Agron.* 14, 145–155.
- Blouin, J., Guimberteau, G., 2003. *Maduración y madurez de la uva*. Mundi-Prensa Libros, pp. 32–40.
- Bonada, M., Jeffery, D.W., Petrie, P.R., Moran, M.A., Sadras, V.O., 2015. Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Aust. J. Grape Wine Res.* 21, 240–253.
- Bowes, G., 1993. Facing the inevitable – plants and increasing atmospheric CO₂. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44, 309–332.
- Castellarin, S.D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., Di Gaspero, G., 2007. Transcriptional regulation of anthocyanins biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell Environ.* 30, 1381–1399.
- Ceulemans, R., Mousseau, M., 1984. Effects of elevated atmospheric CO₂ on woody plants. *New Phytol.* 127, 425–446.
- Cohen, S.D., Tarara, J.M., Kennedy, J.A., 2008. Assessing the impact of temperature on grape phenolic metabolism. *Anal. Chim. Acta* 621, 57–67.
- Collins, D.B.G., Bras, R.L., 2007. Plant rooting strategies in water-limited ecosystems. *Water Resour. Res.* 43 (6), W06407.
- Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S., Geros, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *J. Agric. Food Chem.* 1, 1–22.
- Coombe, B., McCarthy, M., 2000. Dynamics of grape berry growth and physiology of ripening. *Aust. J. Grape Wine Res.* 6, 131–135.
- Cure, J.D., Acock, B., 1986. Crop responses to CO₂ doubling: a literature survey. *Agric. For. Meteorol.* 38, 127–145.
- Dai, Z.W., Meddar, M., Renaud, C., Merlin, I., Hilbert, G., Delrot, S., Gomès, E., 2014. Long term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. *J. Exp. Bot.* 65 (16), 4665–4677.
- De Souza, C.R., Maroco, J.P., Dos Santos, T.P., Rodrigues, M.L., Lopes, C.M., Pereira, J.S., Chaves, M.M., 2005. Grape berry metabolism in field-grown grapevines exposed to different irrigation strategies. *Vitis* 44, 103–109.
- Downey, M.O., Dokoozlian, N.K., Krstic, M., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Viticult.* 3, 257–268.
- Esteban, M.A., Villanueva, M.J., Lissarrague, J.R., 1999. Effects of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. *Am. J. Enol. Viticult.* 50, 418–434.
- Etienne, A., Genard, M., Lobit, P., Mbeguie-A-Mbeguie, D., Bugaud, C., 2013. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *J. Exp. Bot.* 64, 1451–1469.
- García-Escudero, E., Martínez, J., López, D., 2011. Control del rendimiento en Tempranillo blanco mediante deshojado y aclareo de racimos. *Vida Rural* 325, 34–40.
- Glories, Y., Augustin, M., 1993. Maturité phénologique du raisin, conséquences technologiques: applications aux millésimes 1991 et 1992. Actes du Colloque «Journée technique du CIVB» 21 Janvier, Bordeaux, 56.
- Glories, Y., 1978. *Recherches sur la matière colorante des vins rouges*. Thèse de doctorat en sciences. Université de Bordeaux II.
- Gonçalves, B., Falco, V., Moutinho-Pereira, J., Bacelar, E., Peixoo, F., Correia, C., 2009. Effects of elevated CO₂ on grapevine (*Vitis vinifera* L.): volatile composition phenolic content, and in vitro antioxidant activity of red wine. *J. Agric. Food Chem.* 57, 265–273.
- He, F., Mu, L., Yan, G.L., Liang, N.N., Pan, Q.H., Wang, J., Reeves, M.J., Duan, C.Q., 2010. Biosynthesis of anthocyanins and their regulation in colored grapes. *Mol. J.* 15, 9057–9091.
- Idso, K.E., Idso, S.B., 1994. Plant responses to atmospheric CO₂ enrichment in the face of environmental constraints: a review of the past 10 years research. *Agric. For. Meteorol.* 69, 153–203.
- Intrigliolo, D.S., Castel, J.R., 2009. Response of *Vitis vinifera* cv. ‘Tempranillo’ to partial rootzone drying in the field: water relations growth, yield and fruit and wine quality. *Agric. Water Manag.* 96, 282–292.
- Irigoyen, J.J., Goicoechea, N., Antolín, M.C., Pascual, I., Sánchez-Díaz, M., Aguirreola, J., Morales, F., 2014. Growth, photosynthetic acclimation and yield quality in legumes grown under climate change simulations: an updated survey. *Plant Sci.* 226, 22–29.
- Jones, G.V., Davis, R.E., 2000. Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. *Am. J. Enol. Vitic.* 51, 249–261.
- Kizildeniz, T., Mekni, I., Santesteban, H., Pascual, I., Morales, F., Irigoyen, J.J., 2015. Effects of climate change including elevated CO₂ concentration, temperature and water deficit on growth water status, and yield quality of grapevine (*Vitis vinifera* L.) cultivars. *Agric. Water Manag.* 159, 155–164.
- Kliwer, W.M., Weaver, R.J., 1971. Effect of crop level and leaf area on growth, composition and coloration of Tokay grapes. *Am. J. Enol. Viticult.* 22, 172.
- Kliwer, W.M., 1965a. Changes in concentration of glucose, fructose, and total soluble solids in flowers and berries of *Vitis vinifera*. *Am. J. Enol. Viticult.* 16, 101–110.
- Kliwer, W.M., 1965b. Changes in the concentration of malates, tartrates, and total free acids in flowers and berries of *Vitis vinifera*. *Am. J. Enol. Viticult.* 16, 92–100.
- Kliwer, W.M., 1966. Sugars and organic acids of *Vitis vinifera*. *Plant Physiol.* 41, 923–931.
- Kliwer, W.M., 1970. Effects of day temperature and light intensity on coloration of *Vitis vinifera* L. grapes. *J. Am. Soc. Hortic. Sci.* 95, 693–697.
- Korkutal, I., Bahar, E., Carbonneau, A., 2011. Growth and yield responses of cv: merlot (*Vitis vinifera* L.) to early water stress. *Afr. J. Agric. Res.* 6, 6281–6288.
- Koundouras, S., Marinos, V., Gkouliti, A., Kotseridis, Y., Van Leeuwen, C., 2006. Influence of vineyard location and vine water status on fruit maturation of non-irrigated cv: Agiorgitiko (*Vitis vinifera* L.). Effects of wine phenolic and aroma components. *J. Agric. Food Chem.* 54, 5077–5086.
- Kuhn, N., Guan, L., Dai, Z.W., Wu, B.-H., Lauvergeat, V., Gomès, E., Li, S.-H., Godoy, F., Arce-Johnson, P., Delrot, S., 2014. Berry ripening: recently heard through the grapevine. *J. Exp. Bot.* 65, 4543–4559.
- López, M.I., Sánchez, M.T., Díaz, A., Ramírez, P., Morales, J., 2007. Influence of a deficit irrigation regime during ripening on berry composition in grapevines (*Vitis vinifera* L.) grown in semi-arid areas. *Int. J. Food Sci. Nutr.* 58, 491–507.
- Lakso, A.N., Kliwer, W.M., 1975. The influence of temperature on malic acid metabolism in grape berries: I. Enzyme responses. *Plant Physiol.* 56, 370–372.
- Lecourieux, F., Kappel, C., Lecourieux, D., Serrano, A., Torres, E., Arce-Johnson, P., Delrot, S., 2014. An update on sugar transport and signalling in grapevine. *J. Exp. Bot.* 65, 821–832.
- Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreola, J., Pascual, I., Gomès, E., 2014. Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profile in grapevine berries through transcriptomic regulation. *Plant Cell Physiol.* 55, 1925–1936.
- Martínez-Lüscher, J., Kizildeniz, T., Vučetić, V., Dai, Z., Luedeling, E., van Leeuwen, C., Gomès, E., Pascual, I., Irigoyen, J.J., Morales, F., Delrot, S., 2016a. Sensitivity of grapevine phenology to water availability, temperature and CO₂ concentration. *Front. Environ. Sci.* 4, 48.
- Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreola, J., Pascual, I., Gomès, E., 2016b. Ultraviolet-B alleviates the uncoupling effect of elevated CO₂ and increased temperature on grape berry (*Vitis vinifera* cv. Tempranillo) anthocyanin and sugar accumulation. *Aust. J. Grape Wine Res.* 22 (1), 87–95.
- Matthews, M.A., Anderson, M.M., 1989. Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficits. *Am. J. Enol. Viticult.* 40, 52–60.
- Matthews, M.A., Anderson, M.M., Schultz, H.R., 1987. Phenologic and growth responses to early and late season water deficits in Cabernet Franc. *Vitis* 26, 147–160.

- Matthews, M., Ishii, R., Anderson, M., O'mahony, M., 1990. Dependence of wine sensory attributes on vine water status. *J. Sci. Food Agric.* 51, 321–335.
- McCarthy, M.G., 1997. The effect of transient water deficit on berry development of cv: Shiraz (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 3, 102–108.
- Mekni, I., 2014. Grapevine and Climate Change: Response of Two Varieties of Tempranillo (Red and White). Master Thesis. IAMZ-University of Lleida, Spain.
- Mira de Orduña, R., 2010. Climate change associated effects on grape and wine quality and production. *Food Res. Int.* 43, 1844–1855.
- Morales, F., Pascual, I., Sánchez-Díaz, M., Aguirreolea, J., Irigoyen, J.J., Goicoechea, N., Antolín, M.C., Oyarzun, M., Urdiain, A., 2014. Methodological advances: using greenhouses to simulate climate change scenarios. *Plant Sci.* 226, 30–40.
- Morales, F., Antolín, M.C., Aranjuelo, I., Goicoechea, N., Pascual, I., 2016. From vineyards to controlled environments in grapevine research: investigating responses to climate change scenarios using fruit-bearing cuttings. *Theor. Exp. Plant Physiol.* 28, 171–191.
- Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, H., 2007. Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945.
- Mullins, M.G., 1966. Test-plant for investigations of the physiology of fruiting in *Vitis vinifera* L. *Nature* 209, 419–420.
- Myburgh, P.A., 2003. Responses of *Vitis vinifera* L. cv. Sultanina to water deficits during various pre- and post-harvest phases under semi-arid conditions. *S. Afric. J. Enol. Vitic.* 24, 25–33.
- Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., Deloire, A., 2002. Influence of pre and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitecult.* 53, 261–267.
- Ollat, N., Geny, L., Soyer, J., 1998. Les boutures fructifères de vigne: validation d'un modèle de développement de la physiologie de la vigne. I. Caractéristiques de l'appareil végétatif. *J. Int. Sci. Vigne Vin.* 32, 1–9.
- Pastore, C., Dal Santo, S., Zenoni, S., Movahed, N., Allegro, G., Valentini, G., Filippetti, I., Tornielli, G.B., 2017. Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Front. Plant Sci.* 8, 929.
- Petrie, P.R., Cooley, N.M., Clingeleffer, P.R., 2004. The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera* L.) in the current and following season. *Aust. J. Grape Wine Res.* 10, 203–215.
- Poorter, H., 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetation* 104, 77–97.
- Rawson, H.M., Gifford, R.M., Condon, B.N., 1995. Temperature-gradient chambers for research on global environment change. 1. Portable chambers for research on short-stature vegetation. *Plant Cell Environ.* 18, 1048–1054.
- Rebelein, H., 1973. Rapid quantitative determination of tartaric acid. *Chem. Mikrobiol. Technol. Lebensm.* 2, 33–38.
- Ribéreau-Gayon, J., Stronestreet, E., 1965. Le dosage des anthocyanes dans le vin rouge. *Bull. Soc. Chim.* 9, 2649–2652.
- Ribéreau-Gayon, J., Peynaud, E., Sudraud, P., Ribéreau-Gayon, P., 1972. *Traité d'Enologie sciences et techniques du vin. Tome I – analyse et control des vins.* Dunod, Paris.
- Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A., 2006. *Handbook of Enology: The Microbiology of Wine and Vinifications*, vol. 1., 2nd ed. Wiley & Sons, Chichester, UK.
- Ruffner, H.P., Hawker, J.S., Hale, C.R., 1976. Temperature and enzymic control of malate metabolism in berries of *Vitis vinifera*. *Phytochemistry* 15 (12), 1877–1880.
- Sadras, V.O., Moran, M.A., 2012. Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Aust. J. Grape Wine Res.* 18, 115–122.
- Salazar Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Morales, F., 2010. Effects of climate change scenarios on Tempranillo grapevine (*Vitis vinifera* L.) ripening: response to a combination of elevated CO₂ and temperature and moderate drought. *Plant Soil* 337, 179–191.
- Salazar-Parra, C., Aranjuelo, I., Pascual, I., Erice, G., Sanz-Sáez, A., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Araus, J.L., Morales, F., 2015. Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv Tempranillo) grown under simulated climate change (elevated CO₂, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. *J. Plant Physiol.* 174, 97–109.
- Salon, J.L., Chirivella, C., Castel, J.R., 2005. Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. *Am. J. Enol. Vitecult.* 56, 1–8.
- Schultz, H.B., 2000. Climate change and viticulture: a European perspective on climatology: carbon dioxide and UV-B effects. *Aust. J. Grape Wine Res.* 6, 1–12.
- Shellie, K.C., 2006. Vine and berry response of Merlot (*Vitis vinifera* L.) to differential water stress. *Am. J. Enol. Vitecult.* 57 (4), 514–518.
- Spayd, S.E., Tarara, J.M., Mee, D.L., Ferguson, J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitecult.* 53, 171–182.
- Sweetman, C., Sadras, V.O., Hancock, R.D., Soole, K.L., Ford, C.M., 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988.
- Torres, N., Goicoechea, N., Morales, F., Antolín, M.C., 2016. Berry quality and antioxidant properties in *Vitis vinifera* cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. *Crop Pasture Sci.* 67, 961–977.
- Torres, N., Goicoechea, N., Antolín, M.C., 2017. Flavonoid and amino acid profiling on *Vitis vinifera* L. cv Tempranillo subjected to deficit irrigation under elevated temperatures. *J. Food Compos. Anal.* 62, 51–62.
- Tubiello, F.N., Donatelli, M., Rosenzweig, C., Stockle, C.O., 2000. Effects of climate change and elevated CO₂ on cropping systems: models predictions at two Italian locations. *Eur. J. Agron.* 13, 179–189.
- Vidal, M., Blouin, J., 1978. Dosage colorimétrique rapide de l'acide tartrique dans les moûts et les vins. *Rev. Fr. Œnol.* 16, 39–46.
- Walker, A.R., Lee, E., Bogs, J., McDavid, D.A.J., Thomasand, M.R., Robinson, S.P., 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. *Plant J.* 49, 772–785.
- Williams, L.E., Matthews, M.A., 1990. Grapevine. In: Stewart, B.A., Nielsen, D.R. (Eds.), *Irrigation of Agricultural Crops*, Agronomy Monograph No. 30. ASA-CSSA-SSSA, Madison, pp. 1019–1055.
- Yamane, T., Jeong, S.T., Goto-Yamamoto, N., Koshita, Y., Kobayashi, S., 2006. Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Vitecult.* 57, 54–59.
- Zarrouk, O., Francisco, R., Pinto-Marijuan, M., Brossa, R., Santos, R.R., Pinheiro, C., Costa, J.M., Lopes, C., Chaves, M.M., 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. *Agric. Water Manag.* 114, 18–29.